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Effect of Acetylsalicylic Acid in Mitigating Biotic Stress in Grass (*Panicum maximum*)

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Climate change and increasing biotic stressors pose a significant threat to the productivity and quality of tropical forage systems. This study evaluated the effect of foliar application of acetylsalicylic acid (ASA) on agronomic and nutritional parameters of *Panicum maximum* grown under tropical conditions in Orellana province, Ecuador. A randomised complete block design was employed with four treatments: Control (0 L/ha-^{1}), T1 (0.5 L/ha^{-1}), T2 (1.0 L/ha-^{1}), and T3 (1.5 L/ha^{-1}) of ASA, applied every 35 days over an 8-month period. Plant height, herbage mass, and tiller density were measured periodically, and forage samples were collected for bromatological analysis. ASA application significantly enhanced plant performance. The highest dose (T3) increased plant height by 13%, herbage mass by 56% (5751 vs. 2534 kg/ha), and tiller density by 22% compared to the control ($P < 0.05$); Regarding nutritional quality, crude protein content improved from 10.0% in the control to 13.0% in T3. Organic matter content also increased in treated plots, while ash content showed a slight reduction. However, structural fiber fractions remained high across all treatments (neutral detergent fiber [NDF] > 70%; acid detergent fiber [ADF] > 40%), which may restrict voluntary intake and digestibility in ruminants. Despite this limitation, the improved protein content and biomass yield suggest that ASA foliar application can enhance forage value in tropical environments. These findings support ASA as a cost-effective and eco-friendly strategy for mitigating biotic stress and promoting sustainable forage production in dairy systems. Further research is recommended to assess the consistency and duration of these effects under varying environmental and management conditions.
Keywords: Acetylsalicylic acid, biotic stress, *Panicum maximum*, forage quality, forage yield, sustainable livestock systems.

INTRODUCTION

According to FAO (2021) the global population is projected to reach 9.7 billion by 2050. (Gilbert et al., 2021; Mazet et al., 2009), so the great global challenge is to produce food to meet the population's food demands (Rojas-Downing et al., 2017; Uddin & Kebreab, 2020). Data from FAOSTAT (2022) has reported that pasture and forage crops cover 26% of the land area, constituting 70% of the world's land area, and therefore represent the main component of the diet for ruminants (Capstaff & Miller, 2018; Waghorn et al., 2012). In this context, livestock grazing systems have great repercussions on ecological, productive as well as socio-economic aspects (Landau et al., 2000; Michalk et al., 2019; Smit et al., 2021; Uddin & Kebreab, 2020). In fact, according to Rosegrant et al. (2009), livestock products account for 17% of the world's calorie consumption, while protein accounts for 33%. Consequently, according to projections reported by Britt et al. (2018), global milk production is expected to increase from 664 million tonnes (in 2006) to 1,077 million tonnes (in 2050), while meat production is expected to double from 258 million tonnes to 455 million tonnes (FAO, 2018).

On the other hand, a serious concern of the global scientific community is the adverse effects due to greenhouse gas (GHG) emissions (IIPC, 2019). According to Gerber et al. (2013) the livestock sector contributes 14.5% of global GHG

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emissions, with potential impacts on soil, air, and water. Thus, climate change has increased precipitation rates, increased intensity of floods, storms and forest fires, and even the emergence of new diseases for crops, livestock and humans (Olivera Viciedo et al., 2021; Pinto et al., 2008; Uddin & Kebreab, 2020). In the case of tropical pastures (C_4), despite being more tolerant to adverse environmental conditions (Baruch, 1994; Wong et al., 1985), possibly because they have a different metabolic pathway (Moore & Mott, 1973) than temperate pastures (C_3), preliminary studies have shown a decrease in yields, possibly due to the effect of biotic stressors (FAO, 2022; Flachowsky et al., 2013; Guamán-Rivera et al., 2024; Guamán Rivera et al., 2023; Moore et al., 2020). Biotic stress in forages includes damage caused by herbivorous insects, fungal and bacterial diseases, nematodes, and weed competition, which collectively compromise plant health, reduce biomass yield, and diminish nutritional value (Moore et al., 2020). These stressors disrupt physiological processes, hinder nutrient uptake, and may lead to significant stand losses, particularly in tropical environments where high temperature and humidity create optimal conditions for pathogen and pest proliferation. Acetylsalicylic acid (ASA) is a chemical compound known for its anti-inflammatory properties and its role in mitigating biotic stress in plants (Gutiérrez-Coronado et al., 1998; Park et al., 2023; Quintanilla et al., 2020). The mechanisms through which ASA exerts its effects are multifaceted, involving the modulation of plant growth, the activation of defence responses, and the regulation of stress signalling pathways (González-Correa et al., 2007). Our research team conducted a study on the effect of acetylsalicylic acid (ASA) on temperate pastures, reporting interesting results on yield and composition (Guamán-Rivera & Baldeon, 2025). While the present study evaluates in tropical pastures the effect of acetylsalicylic acid as a measure to mitigate biotic stressors.

MATERIALS AND METHODS

***Area study*:** The present study was carried out in the parish of San José de Guayusa, located in the canton of Francisco de Orellana, province of Orellana. The study area has an area of 50,602.10 ha, while the temperature varies from 18 to 36°C.

***Experimental conditions*:** The *Panicum maximus* grass established more than 10 years ago (2015), was sown at a rate of 8 kg/ha^{-1} and fertilised with 150 kg N/ha^{-1} every 6 months. Laboratory analysis of the soil showed 8% organic matter, 5.1 ppm phosphorus (P), 3.24 ppm potassium (K) and 55% base saturation. The texture of these soils is characterised as clay loam.

Experimental treatments: For the development of this work, a total area of 1,155 m^2 was used, which was divided in four experimental plots of 12.5 × 7.50 m. Subsequently, under a completely randomised block design, the following treatments were assigned: Control, no application of SA; T1, 0.5 litre/ha^{-1} of SA; T2, 1 litre/ha^{-1} of SA; and T3, 1.5 litre/ha^{-1} of SA. The experiment had a duration of 8 months, therefore, the doses according to each treatment were applied with a manual sprinkler 7 days after each cut.

***Variable measurements*:** The harvest intervals of *Panicum maximum* was every 35 days, during which time the height of the plant was recorded (Guamán Rivera et al., 2023) using a 1 × 1 quadrant and a flexometer from the basal surface of the plant to the terminal half of the leaf, to determine an average height. Whereas, the amount of biomass (HM, kg ha^{-1}) was obtained using the double sampling technique described by the NRC (1962). Similarly, base cover was estimated with the double sampling method by direct observation where (1), < 20% = very low, (2), 20 to 39% = low, (3), 40 to 59% medium, (4), 60 to 79% high and (5), 80 to 100% very high (Onyeonagu & Asiegbu, 2013). Likewise, the stem/leaf ratio was determined by manually separating each cut.

For chemical composition, grass samples (0.5 kg) of *Panicum maximum* were collected from each treatment to realise chemical determinations in duplicate according to the (AOAC, 2000) procedures. The dry matter (DM) content at 60 and 90°C was determined in a forced air oven for 48 hours, while ash was determined at 550°C for 5 hours (AOAC, 2000). Protein was determined by the Kjeldahl method (Kjeltec Auto 1030 Analyzer, Tecator, Höganäs, Sweden) using CuSO4 /Se as catalyst (Bradstreet, 1954). Finally, the proportion of structural carbohydrates (NDF, FAD and lignin) according to Van Soest et al. (1991) with the addition of sodium sulphite and α-amylase in a semi-automatic apparatus (F800 Fiber Analyzer, Hanon Advanced Technology Group, China).

***Statistical analysis*:** All data were entered and coded in Excel spreadsheets. Afterwards, after a normality check (PROC UNIVARIATE) in the statistical software SAS v.9.4 (SAS Institute Inc., Cary, NC, USA), they were subjected to parametric tests. Under a linear mixed model (PROC MIXED), our study considered as main effects: treatments, period, as well as the interaction Treatment × period, with blocks and residual error being random effects. Means are presented as least squares, which were separated with the PDIFF option of SAS and compared with a Dunnett's test. Significant differences are reported at $P < 0.05$.

RESULTS

Table 1 shows the main agronomic variables determined. Except for basal cover which did not vary between treatments (46 ± 2.6 %, on average). The agronomic responses increased as a function of SA dose (P = 0.001 to 0.026; Table 1). This study shows that the application of 1.5 litres of SA/ha^{-1} to *Panicum maximum* grass increased plant height by 13% compared to the Control, which translates into a 56% increase in the amount of herbage mass (5751 vs. 2534 ± 512 kg/ha^{-1}). Similarly, as expected, the experimental period conditioned

Table 1. Agronomic variables of *Panicum maximum* by the effect of SA.

Item	Treatments				Period									P = value		
	Ctrl	T1	T2	T3	1	2	3	4	5	6	7	8	SEM	Trat	P	T×P
Plant height, cm	63^{c}	70^{b}	73^{a}	73^{a}	61	80	90	53	40	61	88	88	1,5	0,001	0,001	0,990
Botanical composition, %	63^{c}	70^{b}	73^{a}	73^{a}	61	80	90	53	40	61	88	88	1,5	0,001	0,001	0,990
Plant cover, %	47	47	43	47	47	54	62	37	30	40	50	50	2,6	0,302	0,001	0,990
N of tillers, /m^{2}	7^{b}	9^{a}	8^{a}	9^{a}	5	5	7	7	6	10	13	13	0.5	0,008	0,001	0,950
N- de stems/tillers m^{2}	26^{ab}	28^{a}	25^{c}	30^{a}	6	20	31	33	33	30	31	31	2	0,002	0,001	0,960
Herbage mass, kg ha^{-1}	2534^{d}	3475^{c}	4275^{b}	5751^{a}	3200	3211	3552	3835	4050	4112	4523	4817	512	0,026	0,033	0,055
DM, kg ha^{-1}	617^{e}	808^{d}	1250^{b}	1288^{a}	1094^{c}	1125	1132	1156	1245	1224	111	888	61	0,001	0,020	0,001

Treatment: Control, without SA, T1, 0.5 litre/ha^{-1}; T2, 1 litre/ha^{-1}; T3, 1.5 litre/ha^{-1}; SEM, standard error of the mean; a-e Means with different letters in the same row differ at P < 0.05.

Table 2. Chemical composition of *Panicum maximum* by the effect of SA.

	Treatment				Period			P = value		
Items	Control	T1	T2	T3	1	2	SEM	Trat	P	T × P
DM, 60°C	24^{a}	23	24^{a}	24^{a}	24^{a}	24^{a}	0,5	0,730	0,57	0,78
DM, 103°C	22^{a}	20^{c}	21^{b}	21^{b}	21^{a}	21^{a}	0,4	0,070	1.00	1.00
Ash, %	15	14	13	14	14^{a}	14^{a}	1,0	0,420	0,63	0,99
Organic matter, %	85^{b}	86^{a}	87^{a}	86^{a}	86^{a}	86^{a}	1,0	0,630	0,43	0,99
Ethereal extract	18^{a}	17^{b}	17^{b}	16^{c}	17^{a}	17^{a}	0,5	0,180	0,67	0,99
Fibre %	37^{c}	38^{b}	39^{a-b}	40^{a}	39^{a}	38^{a}	0,1	0,001	0,09	0,99
NDF %	70^{b}	70^{b}	71^{a}	71^{a}	69^{a}	70^{a}	0,8	0,007	0,06	0,99
ADF %	38^{c}	38^{c}	39^{b}	40^{a}	39^{a}	39^{a}	0,1	0,001	0,09	0,99
Crude protein %	10^{c}	12^{b}	13^{a}	13^{a}	12^{a}	12^{a}	0,1	0,010	0,19	0,99

Treatment: Control, without SA, T1, 0.5 litre/ha^{-1}; T2, 1 litre/ha^{-1}; T3, 1.5 litre/ha^{-1}; SEM, standard error of the mean; $^{a-e}$ Means with different letters in the same row differ at P < 0.05.

all the agronomic variables evaluated (P = 0.001 to 0.02; Table 1). It is observed that all variables gradually increased as the study progressed, but a concomitant decrease in DM quantity is reported, which could be explained in part by the water deficit of the last 45 days of evaluation. While analysing the interaction of the main factors, differences were detected in the amount of herbage mass and DM (P = 0.001 to 0.055; Table 1).

Chemical composition data of *Panicum maximum* grass by SA effect are indicated in Table 2. The dry matter content was below 30%, as observed in previous studies (24 ± 0.5 %, on average; P = 0.73). However, higher organic matter contents were observed for the T2 treatment (1 litre/ha^{-1}) when compared to the control (85 vs. 87 ± 1%; P < 0.001), although similar to the other SA doses applied (P = 0.35). Similarly, this study showed higher crude protein contents in all treatments that received SA (12.5 ± 0.1%, on average; P = 0.55) which, when compared to the control (Table 2), were found to be highly significant (12.5 vs. 10.0 ± 0.1%; P < 0.001; Table 2). Regarding structural carbohydrate contents, despite the significant differences observed between treatments (P = 0.001 to 0.007), in the case of *Panicum maximum*, according to our study, high contents of NDF (> 70 ± 0.8%) as well as ADF (> 39 ± 0.1%) were surprisingly high, which could condition both feed intake and digestibility of the grasses, respectively. Whereas, the period only showed statistical trends for NDF contents (P = 0.06), but no interaction was detected in the chemical determinations of *Panicum maximum* grass.

DISCUSSION

More recent estimates have reported that climate change is causing significant yield losses associated with more intense droughts due to increased temperatures (FAO, 2022; Olivera Viciedo et al., 2021; Rojas-Downing et al., 2017). According to Kaur et al. (2008) and Mantri et al. (2012) it has been projected that abiotic stresses may adversely affect 70% yield of staple food crops. Whereas, the potential impacts on livestock include changes in production and quality of feed crop and forage (Parsons et al., 2011) water availability (Thornton et al., 2009) animal growth and milk production (Lees et al., 2019) diseases (Gedif Meseret, 2020; Jeon et al., 2014; Wiethoelter et al., 2015) reproduction (Peniche-gonzález et al., 2014) and biodiversity (Clavo Peralta et al., 2022; Fraser et al., 2022; Veintimilla-Ramos et al., 2021). Previous studies in cold climate pastures have reported that the application of SA significantly improved agronomic parameters (Guamán-Rivera & Baldeon, 2025), which reinforces those obtained in our work under tropical conditions. Therefore, depending on the agronomic and compositional results, the mechanisms through which SA exerts its effects are multifaceted, involving modulation of plant growth, activation of defence responses and regulation of stress signalling pathways. The mechanism by which these

responses are enhanced, according to Li et al. (2022) and Khan et al. (2015), is possibly due to activation of the non-expressor of pathogenesis 1 (NPR1) gene and its paralogues, which are essential for SA signalling in plant immunity. Therefore, the results of our research group in both temperate and tropical pastures would provide valuable information that the use of SA could partly mitigate biotic stressors in pastures, resulting in improved yield and compositional responses. Although studies on other plant species have shown that lower concentrations of exogenous SA appear to have a growth-promoting effect, while higher concentrations may negatively regulate growth (Li et al., 2022). Guamán-Rivera & Baldeon (2025) observed that using a dose of 1.5 litres/ha^{-1} improved agronomic parameters, results that coincide with those reported by Ortega-Aguirre et al. (2015) in rice crops. In addition, our work showed a +22% increase in the number of tillers for T3 compared to the Control (7 vs. 9 ± 0.5 tillers/m^2), which justifies the increase in herbage grass (5751 vs. 2534 ± 512 kg/ha^{-1}), probably by modulating cell division (Li et al., 2022). González-Correa et al. (2007) stated that concentrations of 1.5 litre/L of SA, improved vigour the plants presented a greater defence against diseases and pests of this crop.

Crude protein contents at T3 (1.5 litres/ha^{-1}) showed an increase of + 23% compared to those obtained for the control (10 vs. 13 ± 0.1%). According to Givens et al. (2000) forages with crude protein contents below 7% DM were shown to reduce voluntary dry matter intake (DMI). Taking this into account, the CP contents of the HS treatments show values for animals in more demanding stages such as growth or production. We conjecture that high dose of SA improved plant tolerance to heat stress such as reported (He & Liu, 2002). Biotic stress factors can directly affect the crude protein content of grasses, as plants use more resources for defensive mechanisms to cope with such stressors. In this sense, enzymes such as nitrate reductase and glutamine synthetase decrease their activity under stress, reducing the efficiency of nitrogen assimilation. In fact, Seibert et al. (2021) reported to have observed a decrease of about 19% of CP under stress conditions. These results could be compared with ours, since in the control plants we observed contents of − 23% compared to those in which SA was applied. Although fibre is essential for the digestive health of ruminants, excessive fibre content can limit dry matter intake and reduce digestive efficiency (Bruinenberg et al., 2002; Jung & Allen, 1995; Mertens, 2015). Regardless of the treatments, under our conditions, *Panicum maximum* shows both NDF (> 70% in DM) and ADF (> 40% in DM) values above the optimal values for ruminant feed. NDF quantifies the structural components of plant cell walls, including cellulose, hemicellulose, and lignin, while ADF focuses on the less digestible fractions, primarily cellulose and lignin (Raffrenato et al., 2009). McDonald et al. (2010) stated that plants with a high fibre content do not have a good digestibility (Van Soest, 1965). According to Santiago et al. (2013), both components (i.e., NDF and ADF) are inversely related to forage digestibility, also, under biotic stress conditions, it has been observed that their values increase to cope with higher metabolic loads, thus decreasing the nutritive value for grazing animals (Minson, 1990). Consequently, since livestock feeding depends on pasture digestibility, synergies and/or antagonisms between biotic stress and fibre composition are a new front of study that should be further evaluated.

***Conclusion*:** The application of 1.5 L/ha^{-1} of SA on *Panicum maximum* increased biomass production by 56% (5751 vs. 2534 kg/ha) and crude protein content by 23% (13 vs. 10%). These agronomic and bromatological improvements show its potential to mitigate biotic stress under tropical conditions. However, a high fibre content (NDF > 70%) was observed, which could limit digestibility. The number of tillers also increased by 22%, improving pasture density. These results support the use of SA as a sustainable strategy to optimise forage productivity. It is recommended to validate these effects under different soil and climatic conditions.

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